

5 The following oligonucleotides were used in the
construction of plasmids.

Table 2: Oligonucleotides Utilized For LovE Variant Cloning

MO664 (5'GGCCATGGAGGCCGCTAGCTCGAGTCGACGGCCTAGGTGGCCAGCT3')	(SEQ ID NO:1)
MO665 (5'GGCCACCTAGGCCGTCACTCGAGCTAGCGGCCTCATGGCCGTAC3')	(SEQ ID NO:2)
MO666 (5'GGCGGCCGCTCTAGAACTAGTCTCGAGGGTACC3')	(SEQ ID NO:3)
MO667 (5'GGTACCCCTCGAGACTAGTTCTAGAGCGGCCGCC3')	(SEQ ID NO:4)
MO1794 (5'CACAGCGGCCGCTAACCTTCCCATTGGGGC3')	(SEQ ID NO:5)
MO1793 (5'CACCACTAGTACCGGGCTGATTGAC3')	(SEQ ID NO:6)
MO1785 (5'CACCACTAGTTATACATTATAAAGTAATGTG3')	(SEQ ID NO:7)
MO1786 (5'CACAGGATCCGTCATCTTGCCTCGTTATC3')	(SEQ ID NO:8)
MO195 (5'CGCGGATCCTATTGAACAAGATGGATTGCAC3')	(SEQ ID NO:9)
MO196 (5'CCGGAATTCAAGAAGAACTCGTCAAGAAG3')	(SEQ ID NO:10)
MO841 (5'ACAAAAAAAGCAGGCTCCACAATGGCTGCAGATCAAGGTAT3')	(SEQ ID NO:11)
MO842 (5'ACAAGAAAGCTGGTTCATGGAGGAATATTGTTGA3')	(SEQ ID NO:12)
MO2278 (5'GGGGATCCAATCGAGGTCCACGACCACT3')	(SEQ ID NO:13)
MO343 (5'GGGGACAAGTTGTACAAAAAAGCAGGCT3')	(SEQ ID NO:14)
MO2273 (5'GGGGATCCGCCAATGGTCCCCTCAAAC3')	(SEQ ID NO:15)
MO2274 (5'ACAAGAAAGCTGGTTCACAGAATGTTAGCTCAA3')	(SEQ ID NO:16)
MO344 (5'GGGGACCACTTGTACAAGAAAGCTGGGT3')	(SEQ ID NO:17)
MO2624 (5'GCGATGCCCAAGCGCAAGCTACGCCAATCCAGGG3')	(SEQ ID NO:18)
MO2654 (5'CGTCGCCATTGCCATTAGGCTGCGCAACTGT3')	(SEQ ID NO:19)
MO2680 (5'GGACCTTGAGCATAAATTACTATACCTCT3')	(SEQ ID NO:20)
MO2686 (5'GGCGCGTCCATTGCCATTAGGCTGCGCAACTGT3')	(SEQ ID NO:21)
MO2681 (5'TAAAACCTTTCTCTCTCTCTAAAT3')	(SEQ ID NO:22)
MO2700 (5'CAGTGAGCGCGCGTAATACGACTCACTATAGGGCGA3')	(SEQ ID NO:23)
MO2701 (5'ATACTCTATAGACACACAAACACAAATACACACAC3')	(SEQ ID NO:24)
MO107 (5'CGCGGATCCCGTCGTTTACAAC3')	(SEQ ID NO:25)
MO197 (5'CCCAAGCTTATTATTTGACACCAGACCAA3')	(SEQ ID NO:26)
MO1293 (5'GGAAGATCTAGCATCGTGGCCAATTCTTAGTT3')	(SEQ ID NO:27)
MO1294 (5'ATAAGAATGCGGCCGCTAACCTTCCCATTGGGGCGTTGC3')	(SEQ ID NO:28)
MO1787 (5'CACAGGATCCAGCATTATTAATTAGTGTGTATT3')	(SEQ ID NO:29)
MO1788 (5'CACCACTAGTCTCGAGCAGATCCGCCAG3')	(SEQ ID NO:30)
MO1793 (5'CACCACTAGTACCGGGCTGATTGAC3')	(SEQ ID NO:31)
MO1794 (5'CACAGCGGCCGCTAACCTTCCCATTGGGGC3')	(SEQ ID NO:32)
MO511 (5'GGCCATCGATAAGTTGTACAAAAAAGCTGAAC3')	(SEQ ID NO:33)
MO540 (5'GGCGCCCTATTACACCACTTGTACAAGAAAGC3')	(SEQ ID NO:34)
MO1985 (5'CACACGTCTCCGGCCTAACCTTCCCATTGGGGCG3')	(SEQ ID NO:35)

NO:35)	
MO1986	(5' CACACAGATCTCGTGGCCAATTCTTAGTTGA3') (SEQ ID NO:36)
MO1992	(5' CACACGGATCCACAATGTTACGTCTGTAGAAACCC3') (SEQ ID NO:37)
MO1993	(5' CACAGCGGCCGCTTCATTGTTGCCTCCCTGCTG3') (SEQ ID NO:38)
MO316	(5' GCGGCCGCGGCCGCCATGTCAACAAGAAT3') (SEQ ID NO:39)
MO318	(5' CCGCGGCCGAGTGGAGATGTGGAGT3') (SEQ ID NO:40)

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Plasmid MB2254 contains the *lovFp-HIS3p-neo* reporter gene flanked by *URA3* sequence. First primers MO664 (SEQ ID NO:1) and MO665 (SEQ ID NO:2) were annealed and 10 inserted into the *KpnI-SacI* sites of plasmid pBluescript II KS (Stratagene,). The resulting vector, MB1038, contains a *SalI* site in the polylinker. Next, the *SpeI-XhoI* fragment from pJL164 (Brachmann *et al.* *Yeast* 14:115-132 (1998)) containing a deletion of the *URA3* gene with 15 additional flanking sequences was inserted into the *NheI-SalI* sites of MB1038 to create MB1053. Primers MO666 (SEQ ID NO:3) and MO667 (SEQ ID NO:4) that contain multiple restriction sites (*NotI*, *XbaI*, *SpeI*, *XhoI* and *KpnI*) were then annealed together and ligated into the *SmaI* site of 20 MB1053 to create MB1054. Next, the following four fragments were combined in MB1054 to obtain plasmid MB2254. The *lovF* promoter from *A. terreus* genomic DNA was PCR amplified with MO1794 (SEQ ID NO:5) and MO1793 (SEQ ID NO:6) and inserted into MB1054 on a *NotI-SpeI* fragment. 25 The *HIS3* basal promoter from pRS403 (Sikorski and Hieter, *Genetics* 122:19-27 (1989)) was PCR amplified with primers MO1785 (SEQ ID NO:7) and MO1786 (SEQ ID NO:8) and inserted into MB1054 on a *SpeI-BamHI* fragment. Finally, the *neo* gene (PCR amplified with MO195 (*BamHI*) (SEQ ID NO:) and 30 MO196 (*EcoRI*) (SEQ ID NO:10) from plasmid pYX11 (Xiao and Weaver, *Nucl. Acids Res.* 25:2985-2991 (1997)) and *CYC1* terminator sequences (*XhoI-KpnI* fragment from pRS426-GAL-S (Mumberg, *et al.*, *Nucl. Acids. Res.* 22:5767-5768 (1994))) were first combined in pRS416 (Sikorski and Hieter,

5 *Genetics* 122:19-27 (1989)) and then cut out with *Bam*HI-
KpnI and inserted into MB1054 to create MB2254.

The *lovFp-HIS3p-neo* reporter in MY2124 can confer
resistance to the drug geneticin (G418). It was
empirically determined that MY2124 (untransformed or
10 transformed with parental plasmids MB2478 (*CYC1-lovE/CEN*)
or MB2848 (*CYC1-lovE/At274/CEN*) was unable to grow on YPD
media supplemented with 100 µg /ml G418. Plasmid MB2478
contains the *CYC1* promoter operationally linked to the
entire *A. terreus lovE* open reading frame. The *CYC1*
15 promoter is a relatively weak promoter and thus the *lovE*
ORF in MB2478 was expressed at low levels. MB2478 was the
parental vector plasmid for creating full length *lovE*
variants. Plasmid MB2848 contains the *CYC1* promoter
operationally linked to a chimeric open reading frame
20 consisting of the *A. terreus lovE* DNA binding domain fused
to the carboxy-terminal portion of the *At274* gene (U.S.
Serial No. 60/257,431, filed December 22, 2000).

MB2848 was used to create *lovE* variants in which the
DNA binding domain was not mutated. Both MB2478 and
25 MB2848 contain yeast CEN and autonomously replicating
sequences and both are maintained at 1-2 copies per cell.
In contrast to strains transformed with MB2478 or MB2848,
strains transformed with plasmid MB1644 (*TEF1-lovE/2*
micron) were able to grow on G418-supplemented YPD media.
30 The *lovE* gene of MB1644 is under control of the
constitutively strong *S. cerevisiae TEF1* promoter. MB1644
contains a 2-micron origin for high-copy replication in
yeast. An objective of these studies was to identify *lovE*
variants which when expressed at low levels could confer
35 G418 resistance similar to the highly expressed wild-type
lovE molecule of MB1644. *S. cerevisiae* expression vectors
used in these studies were constructed as follows.

MB968 is a low copy *S. cerevisiae URA3* based
expression vector. MB968 was created by inserting the
40 *EcoRV* fragment (containing the destination cassette) from
gateway pEZC7201 (Invitrogen™, Carlsbad, CA) into